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# TRIMETHYLTIN SELECTIVELY ALTERS ACTIVITY OF $Ca^{++}$, $Mg^{++}$, AND $(Ca^{++} + Mg^{++})$-ATPases OF HUMAN NEUROBLASTOMA


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**13. ABSTRACT** *(Maximum 200 words)*
Human neuroblastoma GM3320 tissue homogenates exhibit $Ca^{++}$, $Mg^{++}$, and ($Ca^{++}$ + $Mg^{++}$)-ATPase activities which are ouabain insensitive. Trimethyltin (TMT) discriminates among these three ATPase activities. At low (25 μM) concentrations, TMT stimulated the $Ca^{++}$-ATPase and inhibited the ($Ca^{++}$ + $Mg^{++}$)-ATPase by 70%. At 75 μM, TMT inhibited the $Mg^{++}$-ATPase by 50% and the ($Ca^{++}$ + $Mg^{++}$)-ATPase by 95%. In summary, TMT preferentially inhibited the $Mg^{++}$ and ($Ca^{++}$ + $Mg^{++}$)-ATPase activity of human neuroblastoma GM3320 cells.

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## PREFACE

The work described in this report was authorized under Project No. 1C162706A553C, Decontamination, Detection and Identification. This work was started in October 1986 and completed in November 1986. The experimental data are contained in laboratory notebooks in the Division of Life Sciences, University of Texas at San Antonio (San Antonio, TX).

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TRIMETHYLTIN SELECTIVELY ALTERS ACTIVITY OF $Ca^{++}$, $Mg^{++}$, AND ($Ca^{++}$ + $Mg^{++}$)-ATPases OF HUMAN NEUROBLASTOMA

## 1. INTRODUCTION

Neurotransmitter release from nerve terminals is coupled to the intracellular concentration of $Ca^{++}_i$. The regulation of internal $Ca^{++}_i$ pools is, in turn, accomplished by several processes which include the transport of $Ca^{++}_i$ into mitochondria,[1] the binding of $Ca^{++}$ by intraneuronal or membrane proteins,[2,3] the exchange of intraneuronal $Ca^{++}$ with extracellular $Na^+_o$ via an antiport process,[4,5] and finally, $Ca^{++}$ extrusion or sequestration by $Ca^{++}_i$-activated adenosine triphosphatases (ATPases).[6-9] These processes are sensitive targets for a wide range of neurotoxic compounds with industrial, agricultural, and military applications. In fact, ($Ca^{++}$ + $Mg^{++}$)-ATPase and an associated ion channel are inhibited by Mojave toxin[10] and activated by maitotoxin,[11] two of the most potent known naturally occurring neurotoxins.

Organotin compounds are biologically active organometals which are used as pesticides, fungicides, anti-fouling paints, plastic stabilizers, and catalysts.[12,13] The short chain alkyltins are water soluble and degrade to relatively non-toxic products, but induce neuropathies in mammals.[14-19] Trimethyltin (TMT), the most potent neurotoxic organotin compound,[20] induces psychological and behavioral disturbances in humans characterized by irritability, memory loss, and mental confusion.[21-23]

Neurochemical effects include alterations of muscarinic and dopaminergic receptor binding in mice,[24] inhibition of neurotransmitter uptake in mouse[25] and rat[19] brain synaptosomes, and inhibition of acetylcholine release under conditions of 20 Hz stimulation in the rat phrenic nerve-hemidiaphragm.[26] The apparent nonspecificity of TMT for neurotransmitter systems and the similarity of its effects to those of ruthenium red, which blocks $Ca^{++}$ entry into mitochondria,[27] suggest that TMT may disturb membrane pumps responsible for the regulation of intracellular $Ca^{++}$. These observations and the fact that triethyltin, a related compound, has general effects on enzymes which use ATP as a substrate,[28-30] provide the rationale for assessing the effects of TMT on endogenase ATPase activities in human neuroblastoma cells.

## 2. MATERIALS AND METHODS

### 2.1 Materials.

Trimethyltin chloride and malachite green were purchased from Aldrich Chemical Company, Milwakee, Wisconsin. Adenosine triphosphate (ATP), ouabain, HEPES buffer, Tris buffer, and glycerol were purchased from Sigma Chemical Company, St. Louis, Missouri. Dulbeco Modified Eagle's media (DME-1X) was purchased from Irvine Scientific, Irvine, California. Fetal Calf Serum (FCS) was purchased from Hyclone Labs, Logan, Utah. Trypsin was obtained from Gibco, Grand Island, Maine. Human neuroblastoma cells (GM3320) were obtained from Coriel Human Genetic Mutant Cell Repository, Camden, New Jersey.

2.2 Cell Culture.

Cells were grown at 37°C in Falcon T-75 flasks containing 10 ml DME-1X supplemented with 10% (v/v) HCS in an atmosphere of 1% $CO_2$. Media was changed every three days. Confluency, approximately $1.0 \times 10^{-7}$ cells, was attained on the 7th or 8th day, at which time the culture flasks were treated for 1 min with a 0.05% trypsin solution containing 0.05% EDTA (w/v). Trypsin action was inhibited by addition of an equal volume of growth medium, followed immediately by centrifugation for 5 min (1500 rpm) at room temperature.

The resulting pellet was washed twice with 0.01 M Tris buffer, pH 8.2, containing 16% (v/v) glycerol. The washed pellet was then homogenized (10 strokes) in a Potter Elvejham glass homogenizer previously chilled to 5°C. The protein was determined by the method of Bradford using bovine serum albumin as a standard.[31]

2.3 Enzyme Assays.

All incubations had a final volume of 2.0 ml containing 150 $\mu$g protein, 0.02 M HEPES buffer, pH 7.2, 100 $\mu$M EGTA, 100 mM KCl, 0.1 mM ouabain, indicated amounts of TMT, and 250 $\mu$M ATP. Prior to the addition of ATP, assay mixtures were preincubated for 5 min at 37°C. Hydrolysis of ATP was allowed to proceed for 10 min at 37°C. Reactions were terminated by addition of 200 $\mu$l 6.0 N HCl at which time 200 $\mu$l aliquots were removed. Released phosphate was monitored spectrophotometrically by the method of Lanzetta and coworkers.[32] $Ca^{++}$-ATPase activity was determined by subtracting basal ATPase activity (no added $Ca^{++}$ or $Mg^{++}$) from activity in the presence of 300 $\mu$M $CaCl_2$ (no $Mg^{++}$). $Mg^{++}$-ATPase activity was the difference between basal and $Mg^{++}$-stimulated (1.0 mM) activity (no $Ca^{++}$). ($Ca^{++}$ + $Mg^{++}$)-dependent ATPase activity represents the difference between total activity ($Ca^{++}$ + $Mg^{++}$) and activity in the presence of $Mg^{++}$ alone. Assay conditions described above were linear with respect to both protein and time. Trimethyltin chloride solutions were prepared fresh in glass distilled water: 25, 50, 75, 100, and 125 $\mu$M final concentrations.

3. RESULTS

As shown in the figure, very low concentrations of TMT significantly depress the $Ca^{++}$, $Mg^{++}$, and ($Ca^{++}$ + $Mg^{++}$)-dependent ATPase activities of neuroblastoma GM3320 homogenates. At 25 $\mu$M, the ($Ca^{++}$ + $Mg^{++}$)-dependent ATPase activity (●-●-●) is inhibited by greater than 65% in contrast to a 45% stimulation of the $Ca^{++}$-dependent, $Mg^{++}$-independent activity (o-o-o); whereas, the $Mg^{++}$-dependent, $Ca^{++}$-independent ATPase activity (x-x-x) was observed to be inhibited by approximately 25%. At 75 $\mu$M, no inhibition of the $Ca^{++}$-dependent, $Mg^{++}$-independent ATPase activity was observed; whereas, the ($Ca^{++}$ + $Mg^{++}$)-dependent activity was inhibited greater than 95%. Only a moderate inhibition of approximately 40% of the $Mg^{++}$-dependent, $Ca^{++}$-independent activity was observed at 75 $\mu$M TMT. Concentration of greater than 75 $\mu$M TMT in the respective assays resulted in increasing inhibition of all activities.

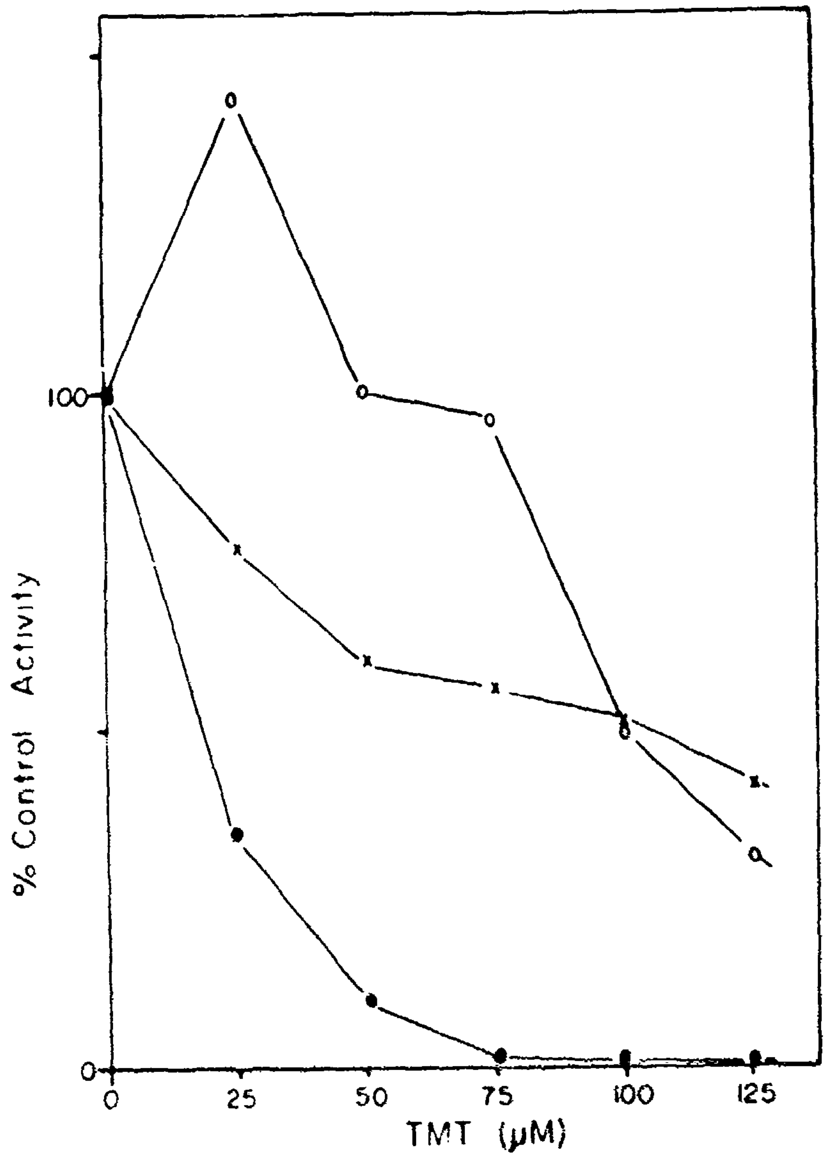


Figure. The effect of increasing amounts of trimethyltin (TMT) on $Ca^{++}$, $Mg^{++}$, and ($Ca^{++}$ + $Mg^{++}$)-dependent ATPase activities of human neuroblastoma GM3320. $Ca^{++}$-dependent, $Mg^{++}$-independent ATPase activity (o-o-o); $Mg^{++}$-dependent, $Ca^{++}$-independent ATPase activity (x-x-x); and ($Ca^{++}$ + $Mg^{++}$)-dependent ATPase activity (●-●-●).

## 4. DISCUSSION

At present, little is known about the mechanism of the neurotoxicity of TMT. A related compound, triethyltin, has been reported to inhibit oxidative phosphorylation and to decrease the incorporation of ($^{14}$C)-glucose into pyruvate and several putative amino acid transmitters.[21] This might account for the observed toxicity to cultured neuroblastoma GM3320 cells of very low concentrations of TMT. Data presented here indicate that TMT may be useful as an activator or inhibitor of ATPase activity in disrupted tissue homogenates. This is consistent with the idea that alkyltins, such as TMT, have a high affinity for membranes and processes associated with membranes as in the inhibition by TMT of the uptake of neurotransmitters into synaptosomes. These results support the contention that this compound may be useful for studying ATP dependent processes such as neurotransmitter release. Recent reports[33,34] suggest that the $Ca^{++}$-ATPase, $Mg^{++}$-ATPase and ($Ca^{++}$ + $Mg^{++}$)-ATPase activities in synaptic plasma membranes reflect the operation of three separate enzymes. Although these activities must be studied in greater detail, their presence suggest the potential usefulness of this cell line in the study of various ATPase dependent neurochemical processes.

## 5. CONCLUSIONS

The $Ca^{++}$, $Mg^{++}$, and ($Ca^{++}$ + $Mg^{++}$)-ATPases are selectively sensitive to perturbation by TMT, suggesting a role for these enzymes in the neuropathologies induced by this organotin. The neuroblastoma GM3320 cell culture provides a convenient system with which to study the mechanisms of action of a wide variety of neurotoxic agents and to assess the potential of these enzymes as detectors of neurotoxins.

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